Immunology: **Keeping mother at bay**

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Like its better-known polymorphic relatives, HLA-G, a relatively unpolymorphic class I MHC molecule expressed by foetal trophoblast cells, binds short peptides with a defined sequence motif; HLA-G may play an important role in maternal tolerance to a foetus.

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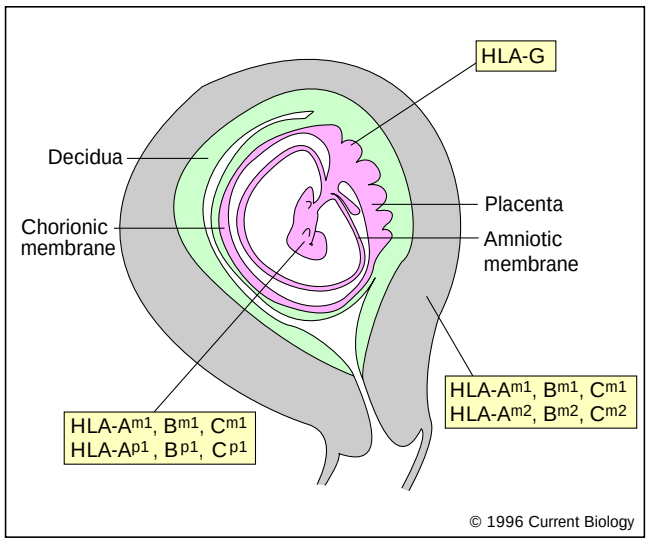
Sixteen years ago, the cloning of the polymorphic class I genes of the human major histocompatibility complex (MHC) — *HLA-A*, *B* and *C* — was driven by a desire to understand how their products carry out the crucial immunological function of presenting antigens to cytotoxic T cells. The DNA probes thus obtained revealed the presence in the human genome of a family of class I MHC genes with at least 15 members in addition to *HLA-A*, *B* and *C*. Almost immediately, the unfamiliar genes became a distinct topic of enquiry, creating a subspecialty based upon ignorance of their associated functions. Within this niche, the foraging strategy for clues about function is to compare the properties of the unanticipated genes and their products with those of *HLA-A*, *B* and *C* and the class I molecules they encode.

Figure 1



The uterus during first trimester, showing the relative positions of cells expressing *HLA-G* and *HLA-A*, *B* and *C*. *HLA-G* is expressed specifically in the extravillous trophoblasts, cells of foetal origin which have invaded the maternal tissues of the placenta. Allotypes of the maternal *HLA* haplotype inherited by the foetus are designated by *m1*, and allotypes of the maternal haplotype not inherited by the foetus by *m2*; allotypes of the paternal *HLA* haplotype inherited by the foetus are indicated by *p1*.

Cloning and sequencing eliminated most of the unanticipated *HLA* genes from further scrutiny, because they were either incomplete or contained stop codons, frame-shifts or other defects that would prevent expression of a functional class I protein. Only three genes passed the preliminary examination [1], and subsequent transfection experiments demonstrated that these genes — *HLA-E*, *F* and *G* — are indeed functional, at least as defined by production in transfected cells of a class I heavy chain that associates with β_2-microglobulin at the cell surface [2]. Although the initial screen selected for genes similar to *HLA-A*, *B* and *C*, tangible differences emerged from further investigation of the successful candidates.

Unlike *HLA-A*, *B* and *C*, *HLA-E*, *F* and *G* have low polymorphism and restricted tissue distribution. One interpretation of these properties is that *HLA-E*, *F* and *G* have specialized functions related to the immunological requirements of the tissues in which they are expressed. This theme, or variations thereof, provides the working hypothesis for much ongoing research in the field. And at this juncture, *HLA-E*, *F* and *G* part company, for their tissue distributions are very different. Most of the limelight has been captured by *HLA-G*, the product of which is almost exclusively found in the extravillous trophoectoderm [3,4], the tissue of foetal origin that directly contacts the maternal circulation during pregnancy and lacks expression of *HLA-A*, *B* and *C* (Fig. 1).

In higher mammals, the development of the embryo within the mother's body can be seen as an adaptation which protects the very young from a hostile, unpredictable environment at a time when they are most vulnerable. In placental mammals, furthermore, the embryo and mother are physically connected, facilitating more efficient communication and disciplined development of the next generation [5]. This familial intimacy occurs commonly in the context of a tissue incompatibility between mother and young that encompasses an entire MHC haplotype, a difference that in other circumstances leads to prompt immunological rejection ('alloreaction'). The structure of the placenta and its environment provides the foetus with protection from the alloreactive tendencies of the mother. In part this privilege is due to physical barriers and selective transport mechanisms, but also implicated

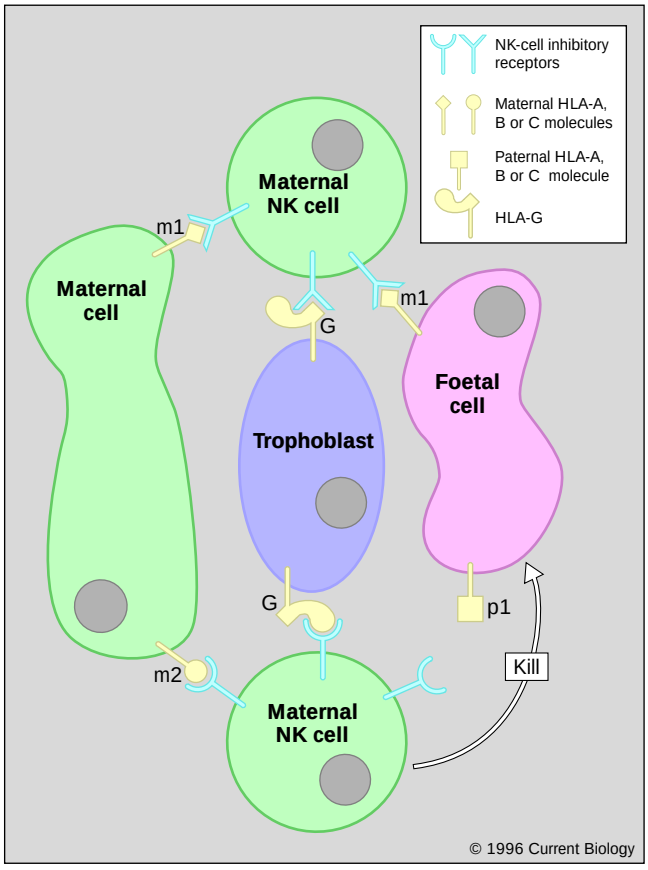
are immunological mechanisms that prevent or suppress the specific immune response. The latter proposition is illustrated by the observation that transgenic female mice expressing T-cell receptors targeted against their mate's MHC class I allotype give birth normally and, during pregnancy, fail to reject subcutaneous tissue grafts of male type. Subsequent to pregnancy, the alloreactive response returns and tissue grafts are then rejected [6].

The lack of polymorphic HLA-A, B and C molecules on extravillous trophoblast probably reduces direct allostimulation of a mother's cytotoxic T-cell response, while expression of monomorphic HLA-G may induce T-cell inactivity in a way perhaps analogous to one of the mechanisms used in generating self tolerance. The presence of many natural killer (NK) cells in the decidua — the maternal tissue contacting trophoblast cells — invites speculation that HLA-G prevents attack of the trophoblast by maternal NK cells [7]. NK cells are innately cytotoxic lymphocytes which are checked from attacking healthy cells by interaction of inhibitory receptors with self class I molecules [8]. NK cells can thus be activated by 'lack of self' [9], as illustrated by the NK-cell-mediated rejection of parental bone marrow by F1 mice [10]. On this principle, those NK cells that in the mother are inhibited by class I molecules of the maternal haplotype not inherited by the foetus have the potential to kill foetal cells and tissues (Fig. 2). Implicit in the speculation that HLA-G molecules prevent NK cells fulfilling their lethal potential is the assumption that HLA-G interacts with cytotoxic T cells and NK cells in a similar manner to HLA-A, B and C. Consistent with this view are experiments showing HLA-G binds the T-cell co-receptor CD8 [11] and mediates inhibition of NK cells [7,12]. Adding further perspective are recent papers in *Current Biology* [13] and *Immunity* [14] which answer the question that has so often dominated recent discussions: does HLA-G bind peptide?

The approach taken by both groups was to purify HLA-G proteins from class I-deficient cells transfected with the *HLA-G* gene, for which HLA-G is the predominant class I species. After denaturation of the HLA-G preparations, released peptides were collected for study. Complicated patterns of peaks were seen when the peptides were separated by reverse phase high-pressure liquid chromatography (HPLC), revealing a heterogeneous pool of peptides, as is characteristic of most HLA class I isoforms. At this point, Lee *et al.* [14] concentrated on determining complete amino-acid sequences for individual peptides within selected peaks from the HPLC pattern. Twelve of the thirteen sequences determined corresponded to nonamer peptides with leucine at the carboxyl terminus (Table 1). Eleven of the peptides had proline at position 3 and sequencing the mixed peptide pool revealed additional selectivity for either arginine or lysine at position 1, and for hydrophobic residues at positions 2 and 7 (Table 1).

Diehl *et al.* [13] obtained results consistent with the binding motif inferred by Lee *et al.* [14]. They also sequenced the peptide pool and three individual peptides extracted from HLA-G (Table 1), and followed this up with a peptide-binding assay. The results showed that residues 2, 3 and 9 of the nonamer peptides serve as 'anchors' for binding to HLA-G. The anchor residue for position 2 is either isoleucine or leucine, at position 3 it is proline, and at position 9 it is leucine. They found some cross-reactivity between peptide binding to HLA-G and to HLA-A2. It is clear from these two studies that HLA-G binds peptides, and that it does so with a similar sequence specificity to the polymorphic HLA molecules.

Figure 2



A possible mechanism by which HLA-G of the trophoblast might protect foetal tissues expressing HLA-A, B and C from attack by maternal NK cells in the decidua. Two subsets of maternal NK cells are defined according to whether they are inhibited by HLA-A, B or C allotypes of one maternal HLA haplotype (m1) or the other (m2). Maternal cells are equally effective at inhibiting both subsets of NK cells, whereas foetal cells inhibit only the NK subset with receptors specific for the m1 haplotype. Foetal cells expressing the paternal haplotype p1 and just one maternal haplotype (m1 in the diagram), cannot inhibit NK cells with inhibitory receptors specific for the other maternal haplotype (m2), and this NK cell subset has the potential to kill the foetus. HLA-G molecules expressed by the trophoblast cells separating the mother and foetus are speculated to engage the inhibitory receptors of all NK cells.

Lee *et al.* [14] went on to show that, also like the polymorphic HLA molecules, cell-surface expression of HLA-G depends on peptide binding. Transfection with *HLA-G* of a cell line lacking the 'TAP' transporter, which delivers peptides to HLA class I molecules in the endoplasmic reticulum, yielded poor levels of cell-surface expression. In cells with TAP, however, HLA-G showed a good level of cell-surface expression and associated transiently with TAP within the endoplasmic reticulum, in a manner similar to that reported for other peptide-dependent class I molecules [15]. In common with transcripts of certain *HLA-A* and *B* alleles, those of *HLA-G* undergo differential mRNA splicing to encode a secreted form of the protein comprising the four extracellular domains, with a similar conformation to the membrane-bound form. Although intracellular association of the soluble molecule with TAP could not be detected, Lee *et al.* [14] found it binds precisely the same set of endogenous peptides as the membrane-bound form.

Now we know for sure that HLA-G needs bound peptides as much as does HLA-A2, the 'man in the street' of the class I allotypes. That the peptide-binding specificity of HLA-G is neither highly specialized nor outside the distribution defined by HLA-A, B and C can only further the notion that the extracellular interactions of HLA-G with other molecules of the immune system are individually analogous to those of HLA-A, B, and C. However, the combined effect of such individual interactions might well be unique; for example, intracellularly, HLA-G has an unusual vestige of a cytoplasmic tail, a potential source of difference for the transduction of signals and functional effects [16]. Overall, the new results reveal the potential for HLA-G to present diverse peptide antigens at the mother–foetus interface, and thereby to stimulate immunity against placental infection. An awaiting challenge is the quest for maternal T cells that respond to HLA-G-presented peptides. That low levels of HLA-G mRNA have been detected in thymus (as well as eye, peripheral blood leukocytes, foetal liver and keratinocytes) certainly leaves open the possibility that clones of cytotoxic T cells are positively selected by HLA-G [17].

Mice do not express HLA-G. A pity, because if nature had been more considerate a knockout mouse with defective *HLA-G* genes would have long been made, and its impact on family values thoroughly explored. All however is not lost, because Schmidt and Orr [17] have seized the opportunity to proselytize mice with HLA-G, and see its effects on their lot. When *HLA-G* is expressed in transgenic mice

Table 1

Endogenous peptides bound by HLA-G.

	Residue number in peptide										
Peptide-binding motif	1	2	3	4	5	6	7	8	9	10	11
HLA-A2		L M							V L		
HLA-G	R K	I L V	P				I L V		L		
Individual peptides											
Ribosomal protein	V	L	P	K	L	Y	V	K	L		
ERP 72	M	R	P	R	K	A	F	L	L		
HLA class III	G	V	P	K	T	H	L	E	L		
HSI protein	M	Q	P	T	H	P	I	R	L		
Fatty acid synthetase	H	V	P	E	H	A	V	V	L		
Unknown	S	Y	P	T	R	I	A	S	L		
Ribosomal protein	K	I	A	G	Y	V	T	H	L		
Cytokine receptor	K	G	P	P	A	A	L	T	L		
Nascent polypeptide	K	S	P	A	S	D	T	Y	I	V	F
Histone	R	I	I	P	R	H	L	Q	L		
Nuclear protein	R	H	P	K	Y	K	T	E	L		
Interferon binding	R	L	P	D	G	R	V	V	L		
Unknown	R	L	P	K	D	F	V	D	L		
Unknown*	R	L	P	K	D	F	R	I	L		
Unknown*	K	L	P	A	Q	F	Y	I	L		
Unknown*	R	I	I	P	R	H	L	Q	L		

In the upper panel, the peptide-binding motifs of HLA-A2 (the A*0201 subtype) and HLA-G are shown. The dominant anchor residues at positions 3 and 9 are shown in bold. In the lower panel, the sequences for individual endogenously bound peptides are shown. The peptides sequenced by Diehl *et al.* [13] are indicated by an asterisk (*); all others were determined by Lee *et al.* [14]. Note that these peptides are those that HLA-G captures with greatest abundance when expressed in Epstein–Barr virus transformed B cells. Although the peptide-binding motif of HLA-G expressed by trophoblast cells is unlikely to change, the repertoire of individual peptides bound may well exhibit differences.

from the *HLA-A2* promoter, its properties resemble those of a conventional class I molecule recognized by the receptors of cytotoxic T cells. Thus, *HLA-G* transgenic skin is rejected by non-transgenic mice, but not by transgenic mice. Such results reaffirm the doctrine that HLA-G is a covert run-of-the-mill antigen-presenting molecule. Setting the stage for future revelations is the fact that, when under the control of its own promoter in transgenic mice, *HLA-G* is expressed in a pattern similar to that of the endogenous gene in humans.

That mice have managed without it for some time (~80 million years) would seem to argue that *HLA-G* does not have a housekeeping function in the biology of placental mammals. Thus, the benefits of having *HLA-G* might well be subtle, perhaps comparable to those conferred by heterozygosity at the *HLA-A*, *B* or *C* loci, and the possible benefits to mice may only be revealed by experiments in which populations of *HLA-G* transgenic and non-transgenic animals are uncaged and pitted one against another. On the human front, a survey of *HLA-G* alleles in African-Americans reveals a previously unsuspected level of polymorphism, and the presence of certain alleles expected to be non-functional [18]. Further assessment of the importance of *HLA-G* could come from expanded study of human populations to determine the frequency of homozygotes for such null *HLA-G* alleles.

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